

## Claims:

1. A single chain T cell receptor (scTCR) comprising
  - 5 an  $\alpha$  segment constituted by a TCR  $\alpha$  chain variable region sequence fused to the N terminus of a TCR  $\alpha$  chain constant region extracellular sequence,
  - a  $\beta$  segment constituted by a TCR  $\beta$  chain variable region fused to the N terminus of a TCR  $\beta$  chain constant region extracellular sequence, and
  - 10 a linker sequence linking the C terminus of the  $\alpha$  segment to the N terminus of the  $\beta$  segment, or vice versa,
  - the constant region extracellular sequences of the  $\alpha$  and  $\beta$  segments being linked by a
  - 15 disulfide bond,
  - the length of the linker sequence and the position of the disulfide bond being such that the variable region sequences of the  $\alpha$  and  $\beta$  segments are mutually orientated
  - substantially as in native  $\alpha\beta$  T cell receptors.
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2. A scTCR as claimed in claim 1 wherein a disulfide bond linking constant region extracellular sequences of the  $\alpha$  and  $\beta$  segments is one which has no equivalent in native  $\alpha\beta$  T cell receptors.
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3. A scTCR as claimed in claim 1 or claim 2 wherein a disulfide bond links amino acid residues in sub-sequences of the  $\alpha$  and  $\beta$  segments corresponding to sequences present in the extracellular constant Ig domains of TCR  $\alpha$  and  $\beta$  chains.
4. A scTCR as claimed in claim 3 wherein the disulfide bond links cysteine
- 30 residues substituted for amino acid residues whose  $\beta$  carbon atoms are less than 0.6

nm apart in corresponding sequences of the extracellular constant Ig domains of TCR  $\alpha$  and  $\beta$  chains

5. A scTCR as claimed in claim 3 or claim 4 wherein the constant region  
5 extracellular sequence present in the  $\alpha$  segment includes a sequence corresponding to the extracellular constant Ig domain of a TCR  $\alpha$  chain, and/or the constant region extracellular sequence present in the  $\beta$  segments includes a sequence corresponding to the extracellular constant Ig domain of a TCR  $\beta$  chain.
- 10 6. A scTCR as claimed in any of the preceding claims wherein (a) the  $\alpha$  segment is the variable region of a TCR fused to the N terminus of the extracellular domain of the  $\alpha$  chain constant region of a TCR  $\alpha$  chain; and/or (b) the  $\beta$  segment is the variable region of a TCR  $\beta$  chain fused to the N terminus of the extracellular domain of the constant region of a TCR  $\beta$  chain.
- 15 7. A scTCR as claimed in any of claims 1 to 5 wherein the constant region extracellular sequences present in the  $\alpha$  and  $\beta$  segments correspond to the constant regions of the  $\alpha$  and  $\beta$  chains of a native TCR truncated at their C termini such that the cysteine residues which form the native interchain disulfide bond of the TCR are  
20 excluded.
8. A scTCR as claimed in any of claims 1 to 5 wherein the constant region extracellular sequences present in the  $\alpha$  and  $\beta$  segments correspond to the constant regions of the  $\alpha$  and  $\beta$  chains of a native TCR in which cysteine residues which form  
25 the native interchain disulfide bond are substituted by another amino acid residue.
9. A scTCR as claimed in claim 8, wherein the said cysteine residues are substituted by serine or alanine.

10. A scTCR as claimed in any of the preceding claims wherein the linker sequence has the formula -P-AA-P- wherein P is proline and AA represents an amino acid sequence wherein the amino acids are glycine and serine.
- 5 11. A scTCR as claimed in any of the preceding claims wherein the linker sequence links the C terminus of the  $\alpha$  domain to the N terminus of the  $\beta$  domain.
12. A scTCR as claimed in claim 11 wherein the linker sequence consists of from 26 to 41 amino acids.
- 10 13. A scTCR as claimed in claim 11 wherein the linker sequence consists of 29, 30, 31 or 32 amino acids.
14. A scTCR as claimed in claim 11 wherein the linker sequence consists of 33, 15 34, 35 or 36 amino acids.
15. A scTCR as claimed in claim 11 wherein the linker sequence is -PGGG-(SGGGG)<sub>5</sub>-P- wherein P is proline, G is glycine and S is serine.
- 20 16. A scTCR as claimed in claim 11 wherein the linker sequence is -PGGG-(SGGGG)<sub>6</sub>-P- wherein P is proline, G is glycine and S is serine.
17. A sTCR as claimed in any of the preceding claims in which an unpaired cysteine residue present in native TCR  $\beta$  chain is not present.
- 25 18. A scTCR as claimed in any preceding claim, wherein the constant region extracellular sequence of the  $\alpha$  segment includes a sequence corresponding corresponds to TRAC\*01 and the  $\beta$  segment includes a sequence corresponding to TRBC1\*01 or TRBC2\*01, and the said non-native disulfide bond is between cysteine residues substituted for Thr 48 of exon 1 of TRAC\*01 and Ser 57 of exon 1 of 30 TRBC1\*01 or TRBC2\*01.

19. A scTCR as claimed in any one of claims 1 to 18, wherein a disulfide bond links cysteine residues substituted for Thr 45 of exon 1 of TRAC\*01 and Ser 77 of exon 1 of TRBC1\*01 or TRBC2\*01.
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20. A scTCR as claimed in any one of claims 1 to 18, wherein a disulfide bond links cysteine residues substituted for Tyr 10 of exon 1 of TRAC\*01 and Ser 17 of exon 1 of TRBC1\*01 or TRBC2\*01.
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21. A scTCR as claimed in any one of claims 1 to 18, wherein a disulfide bond links cysteine residues substituted for Thr 45 of exon 1 of TRAC\*01 and Asp 59 of exon 1 of TRBC1\*01 or TRBC2\*01.
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22. A scTCR as claimed in any one of claims 1 to 18, wherein a disulfide bond links cysteine residues substituted for Ser 15 of exon 1 of TRAC\*01 and Glu 15 of exon 1 of TRBC1\*01 or TRBC2\*01.
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23. A scTCR as claimed in any of the preceding claims, wherein the TCR  $\alpha$  and  $\beta$  chain variable region sequences present in the  $\alpha$  and  $\beta$  segments together correspond to the functional variable domain of a first TCR, and the TCR  $\alpha$  and  $\beta$  chain constant region extracellular sequences present in the  $\alpha$  and  $\beta$  segments correspond to those of a second TCR, the first and second TCRs being from the same species.
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24. A scTCR as claimed in any of claims 1 to 22, wherein the TCR  $\alpha$  and  $\beta$  chain variable region sequences present in the  $\alpha$  and  $\beta$  segments together correspond to the functional variable domain of a first TCR, and the TCR  $\alpha$  and  $\beta$  chain constant region extracellular sequences present in the  $\alpha$  and  $\beta$  segments correspond to those of a second TCR, the first and second TCRs being from different species.

25. A scTCR as claimed in claim 24 wherein the TCR  $\alpha$  and  $\beta$  chain variable region sequences present in the  $\alpha$  and  $\beta$  segments together correspond to the functional variable domain of a human TCR, and the TCR  $\alpha$  and  $\beta$  chain constant region extracellular sequences present in the  $\alpha$  and  $\beta$  segments correspond to those of a mouse TCR.
26. A scTCR as claimed in claims 1 to 24 wherein the TCR is one which binds a peptide MHC complex.
27. A scTCR as claimed in claim 25 wherein the TCR is one which binds a CD1-antigen complex.
28. A scTCR as claimed in claims 1 to 24 wherein the TCR is one which binds a superantigen or a peptide- MHC/superantigen complex.
29. A multivalent T cell receptor (TCR) complex comprising a plurality of sTCRs as claimed in any preceding claim.
30. A scTCR as claimed in any of claims 1 to 28 or a complex as claimed in claim 30 which is covalently linked to a therapeutic agent.
31. A scTCR as claimed in any of claims 1 to 28 or 30, or a plurality thereof, when attached to a particle or bead.
3. A composition comprising a scTCR as claimed in any of the preceding claims and a pharmaceutically acceptable carrier.
33. A method for detecting a TCR ligand selected from MHC-peptide complexes, CD1-antigen complexes, superantigens and MHC-peptide/superantigen complexes which comprises: providing a scTCR as claimed in any one of claims 1 to 29, or a

plurality thereof; contacting the scTCR with the TCR ligand; and detecting binding of the scTCR to the ligand.

34. A method of identifying an inhibitor of the interaction between an scTCR as  
5 claimed in any one of claims 1 to 28, or a plurality thereof, and a TCR ligand selected  
from MHC-peptide complexes, CD1-antigen complexes, superantigens and MHC-  
peptide/superantigen complexes comprising contacting the scTCR with a scTCR  
ligand binding partner, in the presence of and in the absence of a test compound, and  
10 determining whether the presence of the test compound reduces binding of the scTCR  
to the TCR ligand, such reduction being taken as identifying an inhibitor.

35. A method of identifying a potential inhibitor of the interaction between an  
scTCR as claimed in any one of claims 1 to 28, or a plurality thereof, and a TCR  
ligand selected from MHC-peptide complexes, CD1-antigen complexes, superantigens  
15 and MHC-peptide/superantigen complexes comprising contacting the scTCR or  
scTCR ligand binding partner with a test compound and determining whether the test  
compound binds to the scTCR and/or the TCR ligand, such binding being taken as  
identifying a potential inhibitor.

20 36. A nucleic acid molecule comprising a sequence encoding a scTCR as claimed in  
any one of claims 1 to 28, or a sequence complementary thereto.

37. A vector comprising a nucleic acid molecule as claimed in claim 36.

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